Standard Operating Procedure
Air Displacement Pipette Calibration

1 Background:

An accurate pipette is one of the most important tools in performing accurate analytical work. An inaccurate or imprecise pipette will invariable lead to a poor analytical work product, regardless of how much care and skill is used in performing the procedure. With use (and abuse), pipettes will become worn and less reliable. To maintain a valid work product, the state of all pipettes must be periodically checked and verified.

2 References:

2.1 Curtis, RH. Performance reification of manual action pipettes, Part I. Am Clin Lab October 1994.

2.2 Curtis, RH. Performance reification of manual action pipettes, Part II. Am Clin Lab December 1994.

2.3 Eppendorf SOP: Standard Operating Procedure for pipettes. www.eppendorf.com/pipet/SOP

2.4 Clark, John P, Shull, A. Harper. Gravimetric & Spectrophotometric Errors Impact on Pipette Calibration Certainty. Cal Lab, Jan, Feb, Mar 2003.

2.5 The Handbook of Chemistry and Physics, 58th Edition. CRC Press, 1978, p F-11.

3 Scope and Application:

3.1 This procedure describes the procedure for verifying the proper operation and calibration of all makes of air displacement (manual plunger type) pipettes.

4 Method Summary:

Aliquots of Type II water are delivered into a weighing boat, located on a scale, and weighed. If the temperature of the water is well known, the using the density of water will result in the volume of water actually pipetted. The amount actually delivered, compared to the setting on the pipette, results in an indication of the accuracy of the pipette. Repeated weighings for the same setting gives a measure of the precision of the pipette.

5 Sample Handling and Preservation:

5.1 The handling and preservation of a sample is not required in this procedure.

6 Safety:

6.1 There are no safety issues related to this procedure, other than the general safe operating practices for the SASL.

7 Interferences:

7.1 An inaccurate temperature compensation for the density of the water will lead to a systematic error.

7.2 An inaccurate balance will lead to a systematic error.

7.3 Other substances and/or factors not listed above e.g. technical or procedural errors may cause errors.

8 Equipment and Supplies:

8.1 Analytical Balance (Sartorius or equivalent).

8.2 Weighing boats.

8.3 Thermometer.

8.4 500 mL beaker.

8.5 Watch glass to cover the beaker.

9 Reagents:

9.1 No reagents are required for this procedure.

10 Standards:

10.1 Type II Water: A large (approximately 500 mL) quantity should be added to a clean beaker. Cover the beaker with a watch glass, and, using a hot plate, bring the water to a boil. Remove the beaker from the hot plate, and allow the temperature to equilibrate with room temperature for at least two hours before performing this procedure.

11 Procedure:

11.1 On a new Pipette Bench Sheet, record the header information, pipette ID, operating range, water temperature, and balance ID.

11.2 Adjust the pipette to the lowest setting for the operating range of the pipette. Record the setting at the top of the first column on the Bench Sheet.

11.3 With the weighing boat on the balance, tare the balance.

11.4 Using the tips given in Appendix A, carefully pipette an aliquot of the water into the weighing boat.

11.5 Record the weight of the water.

11.6 Tare the balance, and pipette, and record the weight of, another aliquot of water. Repeat the tareing, pipetting, and weighing process until the weights of 30 aliquots have been recorded.

Note: If the pipette is to be used for a single, specialized purpose, it is appropriate to calibrate the pipette for only that volume. The pipette should be calibrated for the desired volume and the decision to calibrate at only one volume should be noted on the Calibration Bench Sheet. The subsequent calibrations should not be performed, and the procedure should be resumed at Section 11.11.

11.7 Change the setting of the pipette to the middle of the range of the pipette.

11.8 Repeat Steps 11.2 - 11.6.

11.9 Change the setting of the pipette to the other extreme of the range of the pipette.

11.10 Repeat Steps 11.2 - 11.6.

11.11 Enter the data into a spreadsheet.

Note: The spreadsheet Pipette.wb3 has been created to perform all of the calculations automatically.

11.12 In the spreadsheet, calculate the volume of each aliquot by applying the correction for the density of water (Equation 13.1).

11.13 Using the spreadsheet, calculate the average and standard deviation of the volumes for each of the aliquots. An example of the spreadsheet is shown in Appendix B.

11.14 Calculate the precision (Equation 13.2) for each volume setting.

11.15 Calculate the Mean Error (Equation 13.3) for each volume setting.

11.16 Calculate the % Mean Error (Equation 13.4) for each volume setting.

12 Quality Control:

12.1 The balance should be calibrated according to the SASL SOP entitled "Analytical Balance Calibration".

12.2 The thermometer should be calibrated according to the SASL SOP entitled "Thermometer Calibration".

12.3 The water should be at least 15 mOhm. The deionizer should be maintained according to the SASL SOP entitled "Deionized Water Care and Maintainance".

12.4 The % Mean Error for each setting should be less than or equal to that given below. If the % Mean Error is greater than that listed below, the pipette fails the calibration.

Volume (µL)	**% Mean Error (Max)**
0.5	5.0
1.01	2.5
5	1.5
10-20	1.0
50-100	1.0
200-1000	0.8
2500-5000	0.6

12.5 The precision for each setting should be less than or equal to that given below. If the precision is greater than that listed below, the pipette fails the calibration.

Volume (µL)	**Precision (Max)**
0.5	3.0
1.02	1.5
5	0.6
10-20	0.5
50-100	0.3
200-1000	0.2
2500-5000	0.2

12.6 If the pipette fails calibration, the pipette should be removed from use until it is serviced and passes a recalibration.

13 Calculations:

13.1 The density of water as a function of temperature:

t / C	**d / gm/mL**
0	0.99987

3.98	1.00000
5	0.99999
10	0.99973
15	0.99913
18	0.99862
20	0.99823
25	0.99707
30	0.99567

Use linear interpolation to approximate the density of the water at temperatures between those shown above. To calculate the density at other temperatures:

$$d = d_1 + (T - T_1) \times \frac{(d_2 - d_1)}{(T_2 - T_1)}$$

where:

d = The density of the water at the desired temperature (gm/mL).
T_1 = The closest temperature below the actual temperature, for which the density is known (°C).
T_2 = The closest temperature above the actual temperature, for which the density is known (°C).
T = The actual temperature (°C).
d_1 = The density for the closest temperature below the actual temperature, for which the density is known (gm/mL).
d_2 = The density for the closest temperature above the actual temperature, for which the density is known (gm/mL).

13.2 Calculate the precision using the Coefficient of Variation (COV):

$$COV = \frac{S \times 100}{\overline{x}}$$

where:

COV = Coefficient of Variation
S = Standard Deviation of the volumes at the setting
= Mean of the volumes at the setting

13.3 Calculate the Mean Error:

$$E = \overline{x} - D$$

where:

E = Mean Error
= Mean of the volumes at the setting
D = The volume setting of the pipette

13.4 Calculate the % Mean Error:

$$\%E = \frac{\overline{x} - D}{D} \times 100$$

where:

%E = % Mean Error
= Mean of the volumes at the setting
D = The volume setting of the pipette

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Revision Log

Revision Number	**Section Revised**	**Effective Date**	**Revision**
1.00	All	10/28/03	Finished version to go to reviewers.

Appendix A
Operating Tips for Air Displacement Pipettes

Volume errors are likely to occur while pipetting liquids that are dense, viscous, or have a high vapor pressure.

Make sure the pipette tip is securely attached.

Always hold the pipette upright. Never lay the pipette down.

The pipette, tips, and liquid should be at the same temperature.

When setting the delivery volume, always approach the desired setting from the high side. If changing the volume to a higher setting, pass the desired setting and then decrease the volume to the desired setting. This procedure eliminates the backlash in the mechanism, allowing for greater reproducibility.

For volumes of greater than 10 µL, pre-rinse (aspirate and dispense the liquid to be pipetted) new tips 2 or 3 times. For volumes of less than 10 µL, do not pre rinse the tip. Pre-rinsing increases precision and accuracy by stabilizing the pressure and temperature of the pipette and wetting the walls of the tip.

For liquids of reasonable viscosity (water and alcohols), reverse pipetting gives the best precision. To reverse pipette, push the plunger past the first detent, put the tip in the liquid, and release the plunger. More liquid will be aspirated than is desired. Dispense the liquid by pushing the plunger down to the first stop, leaving a little liquid in the tip. High viscosity liquids should not be reverse pipetted.

Press and release the plunger slowly and smoothly. Do not just release the button to aspirate the liquid.

While aspirating, keep the plunger a constant and reproducible distance below the surface of the liquid.

When aspirating viscous liquids (blood), leave the tip in the liquid for 1 or 2 seconds after aspirating before removing the tip.

After aspirating, hold the pipette upright while withdrawing the tip from the liquid. Do not hold the tip at an angle or draw the tip against the wall as it is removed from the liquid.

To dispense, hold the tip at an angle against the inside wall of the vessel.